UNIVERSITY OF TECHNOLOGY SYDNEY
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DEVELOPMENT OF A REPORTER FOR PREDICTION OF MEMBRANE FOULING POTENTIAL AND APPLICATION OF NITRIC OXIDE FOR BIOFOULING CONTROL

By

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CERTIFICATE OF AUTHORSHIP/ ORIGINALITY

I certify that the work in this thesis has not been previously submitted for a degree nor has it been submitted as a part of the requirements for other degree except as a fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research and in the preparation of the thesis itself has been fully acknowledged. In addition, I certify that all information sources and literature used are quoted in the thesis.

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ABSTRACT

DEVELOPMENT OF A REPORTER FOR PREDICTION OF MEMBRANE FOULING POTENTIAL AND APPLICATION OF NITRIC OXIDE FOR BIOFOULING CONTROL

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Membrane fouling that results in increases in operational and maintenance costs is a major obstacle to the widespread application of membrane technology [2]. The concentration of assimilable organic carbon (AOC) is directly correlated with the growth of heterotrophic bacteria in water systems. AOC has been widely used as a biofouling indicator for the prediction of biofouling in reverse osmosis (RO) systems [3-5]. In addition to new methodologies to predict fouling, new membrane cleaning technologies are also being developed. One new cleaning method is the application of nitric oxide (NO), which is a biologically active signaling molecule that has been shown to induce biofilm dispersal at nanomolar concentrations [6-9].

In this study, potential AOC reporter strains were isolated for use in desalination RO systems. The results show that strains BLS2, CBSW3 and CBSW4 grew on seawater medium, with maximum cell densities of 5.1 x 10^5, 4.5 x 10^5 and 7.2 x 10^5 colony forming unit (CFU) ml^{-1}, respectively. In addition, all three strains were able to metabolise humic substances which are a major component of AOC in seawater, reaching maximum cell numbers of 5.0 x 10^4 - 2.1 x 10^5 CFU ml^{-1}. With *V. cholerae* A1552 (pUC19-*luxAB*), there was a linear relationship between bioluminescent intensity and glucose concentrations ranging from 0 to 100 µg C L^{-1}, with a coefficient (R^2) of 0.9761. The limit detection was 20 µg C L^{-1}. Our findings provide a more rapid AOC assay, which can quickly determine AOC concentrations within 10 min compared to 30 min performed by *V. fischeri* MJ1 [10, 11].

Pyrosequencing analysis revealed that there was a total of 1,372,739 16S rDNA gene V4 region reads obtained from 9 activated sludge and 10 biofilm samples. RDP Classifier identified 36 phyla, 101 classes and 527 genera of bacteria. The significant changes in relative abundance of the most dominant OTUs associated with the sudden TMP increases demonstrated that species of unclassified *Bacteroidetes*, *Saprospiraceae*, *Comamonadaceae*

and unclassified TM7-3 may play important roles in membrane fouling, and that *Saprospiraceae* and unclassified TM7-3 may be primary colonisers of the membrane. Unclassified *Bacteroidetes* and *Comamonadaceae* (*Comamonas* in particular) may be secondary colonisers of the biofilms on the MBR membranes. Treatment of the membranes with 40 μM DETA NONOate led to a reduction of the TMP by 35% compared to 21% obtained by control distilled water backwash (P value < 0.05), indicating that DETA NONOate was effective in delaying TMP increase.

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ABBREVIATIONS

AFLP: Amplified fragment length polymorphism

AGRF: Australian Genome Research Facility

AOC: Assimilable organic carbon

ASW: Artificial seawater

Atm: Atmosphere

ATP: Adenosine triphosphate

BDOC: Biodegradable dissolved organic carbon

BFM: Berlin filtration method

BioEdit: Biological Sequence Alignment Editor

BLAST: Basic Local Alignment Search Tool

BOD: Biochemical oxygen demand

BOM: Biodegradable organic matter

cAMP: Cyclic adenosine monophosphate

C-di-GMP: Cyclic diguanylate monophosphate

CDOC: Chromatographable dissolved organic carbon

CFU: Colony forming unit

ChromCALC: Chromatography Calculator

COD: Chemical oxygen demand

Da: Daltons

DAP: Diaminopimelic acid auxotroph

DETA: Diethylenetriamine

DGGE: Denaturing gradient gel electrophoresis

DOC: Dissolved organic carbon

EBS: Environmental Business Specialists

EPS: Extracellular polymeric substances

F/M: Food to microorganisms

FISH: Fluorescent in situ hybridization

FMNH2: Flavin mononucleotide

HRT: Hydraulic retention time

KPa: Kilopascal

LB: Luria-Bertani

LC-OCD: Liquid chromatography - organic carbon detection

LMW: Low molecular weight

MAHMA NONOate: 6-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-hexanamine

MBR: Membrane bioreactor

MEGA: Molecular Evolutionary Genetics Analysis

MF: Microfiltration

MFI or $MFI_{0.45}$: Modified fouling index

MIC: Minimal inhibitory concentration

MLSS: Mixed liquor suspended solids

MLVSS: Mixed liquor volatile suspended solids

NCBI: National Center for Biotechnology Information

NF: Nanofiltration

NO: Nitric oxide

NOM: Natural organic material

NTU: Nephelometric turbidity unit

OCD: Organic carbon detection

OLR: Organic loading rate

OND: Organic nitrogen detector

OTU: Operational taxonomic unit

PAOs: Polyphosphate accumulating organisms

PAS: Per-Arnt-Sim

PBS: Phosphate buffered saline

PCoA: Principal coordinate analysis

PCR: Polymerase chain reaction

POC: Particulate organic carbon

PROLI NONOate: 1-(hydroxyl-NNO-azoxy)-L-proline, disodium salt

PVDF: Polyninylidene fluoride

QIIME: Quantitative Insights into Microbial Ecology

QS: Quorum sensing

RDP: Ribosomal database project

RLU: Relative light unit

RO: Reverse osmosis

ROC: Reverse osmosis concentrate

Rpm: revolutions per minute

SCELSE: Singapore Centre for Environment Life Science Engineering

SDI: Silt density index

SIMS: Sydney Institute of Marine Science

SMPs: Soluble microbial products

SNP: Sodium nitroprusside

SRT: Solids retention time

SWRO: seawater reverse osmosis

TMP: Transmembrane pressure

TOC: Total organic carbon

T-RFLP: Terminal restriction fragment length polymorphism

TSS: Total suspended solid

UF: Ultrafiltration

UV: Ultraviolet

UVD: Ultraviolet detector

VFM: VITO fouling measurement

VOCs: Volatile organic chemicals